IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant:	Chiang et al.
Title:	FLUORESCENCE ENERGY TRANSFER BY COMPETITIVE HYBRIDIZATION
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Examiner:	Joyce Tung
Art Unit:	1637
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APPEAL BRIEF

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REAL PARTY IN INTEREST

The real party in interest in this appeal is Quest Diagnostics Investments Incorporated, which is the assignee of the present application.

RELATED APPEALS AND INTERFERENCES

None.

STATUS OF CLAIMS

Claims 1, 10, and 12-13 have been canceled.

Claims 2-9, 11, and 14-38 are pending and under examination in the application.

Claims 2-9, 11 and 14-38 are the subject of this appeal.

STATUS OF AMENDMENTS

The last claim amendments were presented in Appellant's Amendment and Reply Under 37 C.F.R. § 1.111 of January 15, 2009. The claim amendments contained therein have been entered, examined, and are appealed herein. No other amendments or submissions are pending in the application.

SUMMARY OF CLAIMED SUBJECT MATTER

The present invention provides methods for monitoring oligonucleotide production during an amplification reaction. Specifically, the claimed method uses a two probe system in which a first probe contains a fluorophore, a second probe contains a quencher molecule and the two probes are capable of hybridizing in a manner that causes quenching of the fluorescent signal. A target oligonucleotide produced by the amplification reaction, having a sequence complementary to the first probe, competitively hybridizes to that first probe causing separation of fluorophore-containing first probe and the quencher-containing second probe. This competitive hybridization and probe separation results in increased detectable fluorescence from the fluorophore because the fluorescent signal is no longer quenched. Thus, the increase in fluorescence over the course of an amplification reaction may be used to monitor the production of the target oligonucleotide.

Independent claim 20 encompasses a method for real-time monitoring of nucleic acid amplification by amplifying a target nucleic acid and monitoring the nucleic acid in the presence of a first oligonucleotide probe capable of hybridizing to the target nucleic acid and comprising a fluorophore and a second oligonucleotide probe capable of hybridizing to the first oligonucleotide probe and comprising a quencher molecule, wherein the first and second probes are not equal in length and are capable of hybridizing to each other. Amplification of the target nucleic acid is monitored by detecting fluorescence during the amplification reaction, wherein an increase in fluorescence correlates with target nucleic acid amplification. Support for claim 20 is found in the Specification at page 2, lines 1-26, and Figure 1.

Independent claim 23 encompasses a method for monitoring nucleic acid amplification by amplifying a target nucleic acid in a cycling amplification reaction in the presence of a first oligonucleotide probe capable of hybridizing to the target nucleic acid and comprising a fluorophore and a second oligonucleotide probe capable of hybridizing to the first oligonucleotide probe and comprising a quencher molecule, wherein the first and second probes are not equal in length and are cable of hybridizing to each other. Production of the amplified

target nucleic acid is assessed by detecting the amount of fluorescence of the first probe fluorophore during a plurality of cycles of the amplification reaction, wherein the amount of fluorescence correlates with the amount of amplified target nucleic acid. Support for claim 23 is found in the Specification at page 2, lines 1-26, page 9, line 11 to page 10, line 11 (Example 3) and at Figure 1.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Claim 38 stands finally rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the written description requirement.

2. Claims 2-9, 19-29 and 36-38 stand finally rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Heid et al. (Genome Res. 6:986-994 (1996); "Heid") in view of Heller (U.S. Patent No. 5,565,322; "Heller") as evidenced by Mullis et al. (U.S. Patent No. 4,965,188; "Mullis").

3. Claims 11 and 30 stand finally rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Heid et al. (Genome Res. 6:986-994 (1996); "Heid") in view of Heller (U.S. Patent No. 5,565,322; "Heller") as applied to claims 2-9, 19-29, and 36-38, further in view of Di Cesare et al. (U.S. Patent No. 5,716,784; "Di Cesare").

4. Claims 14-18 and 31-35 stand finally rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Heid et al. (Genome Res. 6:986-994 (1996); "Heid") in view of Heller (U.S. Patent No. 5,565,322; "Heller") as evidenced by Mullis et al. (U.S. Patent No. 4,965,188; "Mullis"), as applied to claims 2-9, 19-29, and 36-38, further in view of Hiroaki et al. (EP 0461 863 A1; "Hiroaki").

ARGUMENT

1. Rejection of claims 38 under 35 U.S.C. § 112, first paragraph.

Appellants respectfully traverse the rejection of claim 38 under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the written description requirement.

1.1. The legal standard for written description.

The proper standard for determining compliance with the written description requirement is whether the specification reasonably conveys to the skilled artisan that the inventor was in possession of the claimed invention as of the filing date. See M.P.E.P. § 2163.02 (citing *Ralston Purina Co. v. Far-Mar-Co., Inc.*, 772 F.2d 1570, 227 U.S.P.Q. 177, 179 (Fed. Cir. 1985)). The subject matter of the claimed invention need not be described literally in the specification in order to satisfy the requirements of 35 U.S.C. § 112, first paragraph. Id.

1.2. The Specification discloses monitoring fluorescence of a first probe fluorophore at every cycle during an amplification reaction.

The Examiner wrongly alleges that the Specification does not describe that the fluorescence of said first probe is detected during every cycle of said amplification reaction as recited in claim 38. Appellants respectfully traverse this rejection.

The Specification discloses that the invention relates to a method for monitoring nucleic acid amplification (Specification at page 2, lines 1-2) and that Example 3 shows a real-time PCR experiment using a method of the invention in which fluorescence is monitored during every amplification cycle. The Specification states:

> During PCR, some of the FAM labeled probe and some of the TAMRA labeled probe annealed to the PCR product thus reducing the quenching of the FAM fluorescence and allowed increased fluorescence to be detected. The fluorescence of FAM increase with increasing number of cycles of thermocycling, corresponding with increases in amount of PCR product, as illustrated in Figure 1.

Specification at page 9, lines 21-26 (emphasis added).

Furthermore, the working examples detail an assay in which fluorescence was measured during every cycle. Example 3 provides one such method. Specification at pages 9-10. Specifically, the experimental conditions listed in Table IV (column 4) indicate that the PCR was run for 40 cycles and the results from that experiment, shown in Figure 1, provide individual data points for the fluorescence measured during each of those 40 cycles. Consequently and contrary to the Examiner's allegation, the Specification does disclose monitoring fluorescence of a first probe fluorophore at every cycle during an amplification reaction. Appellants respectfully submit that this rejection is traversed and request that the rejection be reversed and withdrawn.

2. Rejection of claims 2-9, 19-29 and 36-38 under 35 U.S.C. § 103.

Appellants respectfully traverse the rejection of claims 2-9, 19-29 and 36-38 under 35 U.S.C. § 103(a) as allegedly being obvious over Heid et al. (Genome Res. 6:986-994 (1996); "Heid") in view of Heller (U.S. Patent No. 5,565,322; "Heller") as evidenced by Mullis et al. (U.S. Patent No. 4,965,188; "Mullis").

2.1. The legal standard for obviousness.

An invention is unpatentable as obvious if the differences between the patented subject matter and the prior art would have been obvious at the time of invention to a person of ordinary skill in the art. In order to make a *prima facie* case of obviousness, the Examiner must demonstrate that the prior art (i) teaches or suggests every claim limitation, (ii) provides a motivation to combine (or modify) the teachings of the selected references, and (iii) provides a reasonable expectation of success. *In re Vaeck,* 947 F.2d 488, 493, 20 U.S.P.Q. 2d 1438 (Fed. Cir. 1991); M.P.E.P. § 2143. This is the "TSM" test for obviousness which was recently affirmed by the Supreme Court. KSR Int'l Co. v. Teleflex Inc., No. 04-1350, 127 S.Ct. 1727, 1741 (2007). In explicating the correct application of this test, the KSR Court reaffirmed previous holdings that an invention "is not proved obvious merely by demonstrating that each of

its elements was, independently, known in the prior art." Id at 1741, see also, In re Rouffet, 149 F.3d 1350, 1357, 47 U.S.P.Q.2d 1453 (Fed. Cir. 1998). Furthermore, the Court warned the fact finder to be aware of the distortion caused by hindsight bias and to be cautious of arguments reliant upon *ex post* reasoning. KSR, at 1742.

The Federal Circuit has mandated that, when considering the scope and content of the prior art, it is necessary to consider the entire teachings of a prior art reference, not just selected pieces to support an obviousness rejection. In re Fritch, 972 F.2d 1260, 1264, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992) ("[A] prior art reference is relevant for all that it teaches to those of ordinary skill in the art."). "It is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art." Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc., 796 F.2d 443, 448, 230 U.S.P.Q. 416 (Fed. Cir. 1986, quoting In re Wesslau, 353 F.2d 238, 241, 147 U.S.P.Q. 391, 393 (C.C.P.A. 1965). Furthermore, "[a] *prima facie* case of obviousness can be rebutted if the applicant... can show 'that the art in any material respect taught away' from the claimed invention." In re Haruna, 249 F.3d 1327, 1335, 58 U.S.P.Q.2d 1517 (Fed. Cir. 2001), citing In re Geisler, 116 F.3d 1465, 1469, 43 U.S.P.Q.2d 1362, 1365 (Fed. Cir. 1997), In re Malagari, 499 F.2d 1297, 1303, 182 U.S.P.Q. 549, 553 (C.C.P.A. 1974).

2.2. The Combination of Heid et al. and Heller

Claims 2-9, 19-29 and 36-38 stand finally rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Heid et al. (Genome Res. 6:986-994 (1996); "Heid") in view of Heller (U.S. Patent No. 5,565,322) as evidenced by Mullis et al. (U.S. Patent No. 4,965,188; "Mullis"). Appellants respectfully traverse.

Independent claims 20 and 23 encompass methods for the real-time monitoring of nucleic acid amplification (e.g., PCR) by amplifying a target nucleic acid and monitoring the nucleic acid in the presence of two related oligonucleotide probes. The first probe is capable of

hybridizing to the target nucleic acid and contains a fluorophore. The second probe is capable of hybridizing to the first probe and contains a quencher molecule, wherein the detectable signal from the fluorophore is quenched when the probes are hybridized to each other. Amplification of the target nucleic acid is monitored by detecting increased fluorescence during the amplification reaction, wherein the increased fluorescence results from the hybridization of the first probe and the amplified target (i.e., the fluorophore is dequenched).

Briefly, Appellants submit that the fluorogenic probe structures of Heid and Heller are incompatible and their combination, as alleged by the Examiner, would not result in the instant invention. Heid provides a single detection probe for real-time amplification monitoring. This single probe contains both a quencher and a fluorophore which become separated (and fluorescence enhanced) as a result of the exonuclease activity associated with the DNA polymerase used for nucleic acid replication and amplification. In contrast, Heller provides a multi-probe fluorescence resonance energy transfer (FRET) system in which the detectable fluorophore and quencher are on separate probes and the fluorophore receives its excitation energy from a third target-specific probe which contains donor fluorophores. The Examiner has made no credible argument as to how the three-probe FRET system of Heller could be adapted for use in the single probe system of Heid which relies on exonuclease digestion for dequenching. Furthermore, in order to sustain this rejection, the Examiner is cherry picking only a portion of the Heller FRET system (two of the three probes) from the entire teaching, without adequate rationalization or justification. This is unquestionably an impermissible hindsight reconstruction in which the Examiner disregards the context and teachings of the cited prior art references in order to merely identify every claim element. Finally, the Examiner has also disregarded a clear teaching away, previously made of record, based on Tyagi et al.

2.2.1. Heid et al.

Heid fails as a primary reference, and using the probe of Heller in the method of Heid produces a non-functioning result. The Examiner notes that Heid discloses a real time quantitative PCR method in which PCR product accumulation is measured through a dual-

labeled fluorogenic probe. Final Office Action at page 3. However, the Examiner fails to acknowledge that the dual-labeled fluorogenic probe of Heid consists of a single oligonucleotide having a reporter dye (FAM) and a quencher dye (TAMRA) wherein, when the single probe is intact, fluorescent energy transfer occurs and the reporter dye fluorescent emission is absorbed by the quenching dye and no detectable signal results. Heid at page 987, second column. During polymerase extension, the single probe is cleaved by the enzyme's 5' to 3' nucleolytic activity and the two dyes (fluorophore and quencher) are permanently separated. Id. This probe digestion results in an accumulation of the free (dequenched) fluorophore whose detectable fluorescence is proportional to the amount of digested probe and, therefore, the amount of amplified target nucleic acid. Nothing in Heid teaches or suggests how this single probe detection methodology may be modified to a multi-probe system.

The Examiner acknowledges that Heid does not disclose a first fluorophore-containing oligonucleotide probe and a second quencher-containing oligonucleotide probe as recited in independent claims 20 and 23. Final Office Action at page 3. Rather, the Examiner looks to Heller for such disclosure. However, the Examiner fails to demonstrate how the three probe system of Heller may be adapted, in combination with Heid, to arrive at the instant invention or why the artisan would be motivated to make such a combination.

2.2.2. Heller

(1) The Heller Detection System is Incompatible with Heid

The Examiner alleges that Heller provides a nucleic acid detection system having a first probe which contains a fluorophore and is capable of hybridizing to a target nucleic acid, and a second probe, containing a quencher, which is capable of hybridizing to the first probe and quenching the fluorescence of the fluorophore upon hybridization. Final Office Action at pages 3-4. The Examiner further alleges that it is obvious to use this probe pair in the method of Heid. However, in making these allegations, the Examiner ignores the complete detection system of Heller, which comprises three necessary probes, and instead cherry picks only the claim elements

needed to assert against the instant claims, but which functionally destroys the Heller system in the process. This method of analysis is incorrect and clearly represents a hindsight reconstruction made possible only by a wanton disregard for the full teachings and context of the cited prior art.

To sustain this rejection, the Examiner relies on the detection method disclosed in Figure 4 of Heller. Also see, Heller at col. 6, ll. 38-52 and col. 28, ll. 14-39. Here, Heller describes a three probe detection system. The system consists of a first probe containing a fluorophore, a second probe containing a quencher capable of hybridizing to the first probe, and a third probe ("MDO probe") containing multiple donor fluorophores capable of FRET with the fluorophore bound to the first probe. This third probe has a sequence unrelated to the first two (i.e., incapable of hybridization), but is capable of hybridizing to the target nucleic acid at a location that permits FRET to the first probe-bound fluorophore when both are hybridized to the target nucleic acid. Heller repeatedly states that the purpose of this FRET-based detection system is to enhance the detectable signal. Specifically, Heller states:

> It has now been discovered that multiple chromophore donor groups which are located beyond the normal Förster distance (>5 nm) can be arranged to absorb and transfer photonic energy to a terminal acceptor group thereby acting as a light antenna or photonic conductor.
>
> Heller at col. 4, ll. 28-33.

Heller further describes the advantages of this FRET-based system:

> Under conditions where $h\nu_1$ is non-saturating, photonic energy can be collected by arrays of donor groups and directionally transferred to an appropriate acceptor, greatly enhancing its fluorescent emission at $h\nu_2$. This can be considered a molecular antenna or amplifier mechanism.
>
> Heller at col. 4, ll. 28-33.

Thus, it is clear that the third probe containing multiple donor fluorophores, which facilitates signal amplification, is a critical component of the Heller detection system. In asserting this rejection, the Examiner completely and incorrectly disregards this critical element in Heller. A skilled artisan reading Heller would not be motivated to remove this element from the disclosed

detection system as the Examiner has done because such an omission would vitiate the advantages of the entire system.

Furthermore, use of the entire Heller three-probe system in the method of Heid would not result in a functional detection assay. As discussed above, the Heid detection methodology relies on the 5'- to 3'-exonuclease activity associated with the DNA polymerase enzyme used for nucleic acid replication. The exonuclease activity would digest both the first (detector) probe and the third ("MDO") probe upon hybridization to the target nucleic acid. No detectable signal would be produced because the "molecular antenna" would be destroyed and spatially separated from the detectable fluorophore. Thus, the skilled artisan would not combine the detection system of Heller with the detection system of Heid, as alleged by the Examiner because of technical incompatibilities.

(2) There Is No Motivation To Combine Heid With Heller

The Examiner alleges that Heller discloses a method for monitoring nucleic acid during the amplification reaction. Final Office Action at page 3, last paragraph. This is incorrect. In fact, the skilled artisan would not be motivated to look to Heller in order to modify the Heid detection system because each prior art reference is concerned with detecting target nucleic acids under different experimental conditions. As discussed above, Heid is concerned with the real-time monitoring of the accumulation of an amplification product (i.e., under amplification cycling conditions); whereas Heller provides a method for detecting a target nucleic acid under static, non-cycling conditions. It is the fundamental differences between the amplification cycling and static non-cycling conditions that militates against the finding of a motivation to combine these prior art references.

The Examiner has mischaracterized Heller with regard to real-time detection. The Examiner's allegation that Heller provides methods for detecting a target nucleic acid during amplification is incorrect. Rather, Heller is replete with evidence to the contrary. For example, Heller contains no mention of the elements required for an amplification reaction including a

polymerase, deoxynucleotide triphosphates, the use a cycling mechanism characteristic of PCR, or a real-time acquisition of signal during multiple amplification steps.

At column 19, lines 20-56 (relied upon by the Examiner), Heller states:

> Thus a diagnostic method for detecting the presence of a preselected nucleic acid sequence in a nucleic acid-containing sample is contemplated comprising the steps of:
>
> a) admixing:
>
> (i) a polynucleotide having (1) at least two donor chromophores operatively linked to a polynucleotide by linker arms, such that the chromophores are positioned by linkage along the length of the polynucleotide at a donor--donor transfer distance, and (2) at least one fluorescing acceptor chromophore operatively linked to the polynucleotide by a linker arm, such that the fluorescing acceptor chromophore is positioned by linkage at a donor-acceptor transfer distance from at least one of the donor chromophores, wherein the polynucleotide has a nucleotide sequence that is preselected as to be complementary to the preselected "target" nucleic acid sequence; with
>
> (ii) a nucleic acid-containing sample containing the preselected nucleic acid base ("target") sequence to form a hybridization reaction admixture;
>
> (b) subjecting the hybridization reaction admixture to hybridization conditions for a time period sufficient for the polynucleotide to hybridize to the target sequence and form a donor chromophore containing- and acceptor chromophore containing-hybridized nucleic acid duplex;
>
> (c) exciting the donor chromophore in the nucleic acid duplex formed in step (b) by exposing the donor chromophore to sufficient photonic energy to induce emission of photonic energy from the acceptor chromophore; and
>
> (d) detecting the presence of photonic energy emitted from the excited acceptor chromophore, thereby detecting the presence of the preselected nucleic acid sequence in the sample.

Heller at col. 19, ll. 8-40.

In the above detailed methodology, Heller describes no polymerase, no mention of deoxynucleotide triphosphates, no mention to use a cycling mechanism characteristic of PCR, and no mention of real-time acquisition of signal during multiple amplification steps. Based on the total absence of the elements required for real-time nucleic acid detection, this passage can only refer to a *post facto* detection methodology in which the full complement of target nucleic acid is present at the outset of detection.

The Examiner also relies on Heller at col. 21, ll. 28-35 to make the combination with Heid. However, this passage also fails to suggest real-time monitoring of an amplification reaction. Applicant respectfully directs the Examiner to the full context of the cited passage at col. 20, line 57 through col. 21, line 46. Here, Heller describes the various formats in which the disclosed detection methodology may be applicable, including heterogeneous and homogenous formats. Heller at col. 20, ll. 57-67. Heterogeneous formats include an insoluble matrix (e.g., solid support) to which the target nucleic acids are bound; whereas, homogenous formats contain soluble target nucleic acids. Although Heller mentions PCR (a homogeneous format) as a possible source of target nucleic acid, Heller makes clear that the target nucleic acid detection is performed under static, non-cycling conditions. Heller states:

> Where the nucleic acid containing a target sequence is in a double-stranded (ds) form, it is preferred to first denature the dsDNA, as by heating or alkali treatment, prior to conducting the hybridization reaction. The denaturation of the dsDNA can be carried out prior to admixture with a polynucleotide to be hybridized, or can be carried out after the admixture of the dsDNA with the polynucleotide. Where the polynucleotide itself is provided as a double-stranded molecule, it too can be denatured prior to admixture in a hybridization reaction mixture, or can be denatured concurrently therewith the target-containing dsDNA.

Heller at col. 21, ll. 36-46.

It is clear from the context of this passage that, at most, Heller describes only preferred hybridization conditions (i.e., buffer, temperature, etc.) for the above static assay

method. There is no indication that target nucleic acid detection is to occur in real-time during an amplification reaction. In fact, several of the proposed methodologies are inconsistent with real-time detection. For example, in the above passage, Heller suggests the denaturation of dsDNA be performed by alkali treatment. This denaturation method is incompatible with a real-time PCR detection methodology which necessarily uses heat denaturation. Furthermore, Heller suggests that the dsDNA may be denatured prior to admixture with the probe polynucleotide, or that the probe polynucleotide, if double-stranded, may be denatured prior to admixture with the hybridization reaction. These methods are also incompatible with a real-time monitoring system in which all components must be simultaneously present during denaturation and reannealing. This passage is typical of the entire Heller patent in being silent to real-time amplification and the specifics of a real-time assay including failing to mention a polymerase, deoxynucleotide triphosphates, a cycling mechanism characteristic of PCR, or use of real-time acquisition of signal during multiple amplification steps. Nothing in this passage demonstrates any conception of a real-time detection system, as currently claimed.

Finally, the Examiner cites to Heller's example illustrated in Figure 4 and the accompanying text at col. 6, ll. 38-52 and col. 28, ll. 14-39. This example, like the rest of the Heller specification, fails to disclose any real-time amplification monitoring methods. In fact, this example specifically describes a target nucleic acid detection under non-cycling conditions.

In describing this assay system, Heller states:

> FIG 4 shows the homogeneous assay procedure. This procedure can be carried [out] using aqueous buffers common to the art of hybridization... The target DNA is either already present or now added to the system.

Heller at col. 28, ll. 14-22 (emphasis added).

Heller's instruction that "[t]he target DNA is either already present or now added to the system" mandates detection under non-cycling conditions. This instruction is incompatible

with real-time amplification monitoring in which the target DNA, if present, is progressively generated and accumulates over time.

In summary, Heller does not describe, enable, or contemplate a method for monitoring of target nucleic acid during amplification. Throughout the specification, Heller describes detection methodologies only in the context of a static, non-cycling system. There is no disclosure of the elements necessary to perform real-time monitoring. Thus, because of the specialized requirements of real-time monitoring compared to static/non-cycling detection assays, a skilled artisan is not motivated to combine any elements of the Heller detection system with those of Heid, as alleged by the Examiner.

2.2.3. Mullis

The introduction of Mullis does not cure any of the above deficiencies, nor is it cited for that purpose. Mullis merely discloses PCR amplification using a thermostable polymerase and a pair of primers. There is nothing in Mullis that discloses using a multi-probe detection system for real-time PCR, remedies the incompatibilities of Heid and Heller, or otherwise motivate the artisan to combine these prior art references.

2.3. Tyagi et al. Teaches Away From The Claimed Invention

Appellant notes that the instant appeal is the second appeal in this case. Appellant previously appealed a final rejection for obviousness based on Tyagi et al. (U.S. Patent 6,103,476). Following Appellant's Appeal Brief, the Examiner withdrew the case from appeal and reopened prosecution, removing the rejections based on Tyagi et al. As discussed in more detail below and argued in the first Appeal Brief, Tyagi et al. provides a significant teaching away from the claimed invention and the combination of prior art elements currently alleged by the Examiner. Specifically, Tyagi et al. clearly and repeatedly states that bimolecular probes (i.e., two individual probes each containing either a fluorophore or a quencher) are unsuitable for monitoring cycling amplification reactions, such as PCR, in which the bimolecular probes and

targets are repeatedly melted and reannealed. Instead, unimolecular probes (i.e., a single probe containing both a fluorophore and a quencher, such as in Heid) should be used for this purpose. According to Tyagi et al., bimolecular probes are suitable only for non-cycling conditions. The Examiner has never addressed Tyagi's teaching away or provided reasons for why the newly asserted combination of Heid and Heller rebuts it. Instead, the Examiner ignores the teaching away of Tyagi et al. and persists again with an unsustainable rejection. Appellant submits that, even though the Examiner has not relied on Tyagi et al. for the instant rejection, Tyagi's teaching away remains relevant and is dispositive on the issue of the lack of motivation to combine.

Tyagi et al. provide methods for detecting target nucleic acids in a variety of assays, including monitoring the progress of an amplification and other reactions. Tyagi et al. at col. 3, l. 51 through col. 4, l. 21. The methods are based on providing a "unitary probe" which contains (i) a target complementary region, (ii) an affinity pair flanking the target complementary region, and (iii) interactive label moieties. Tyagi et al. at col. 4, l. 59 through col. 5, l. 2. The target complementary region encodes a nucleotide sequence complementary to the target nucleic acid which serves as the basis for target-specific detection. The interactive label moieties are, for example, a fluorophore and quencher pairs. Tyagi et al. at col. 5. ll. 13-26. The affinity pair (e.g., complementary nucleic acid sequences) holds the unitary probe in a "closed conformation" in the absence of target nucleic acid such that the interactive label moieties are held in close physical proximity. The "open conformation" of the unitary probe occurs in the presence of target nucleic acid which disrupts the affinity pair binding, causing physical separation of the interactive label moieties. Tyagi et al. at col. 5, ll. 13-63.

Despite the potentially confusing terminology, Tyagi's "unitary probes" are designed to act as a functional unit, and may be unimolecular or bimolecular. Tyagi et al. at col. 4, ll. 59-62. In a bimolecular construction, the Tyagi probes consists of a pair of oligonucleotides, each containing a different target complementary sequence and an "arm" portion complementary to the arm portion of the other oligonucleotide. Tyagi et al. at col. 5, ll. 3-4. Together, hybridization of the arm portions form a stem which holds the fluorophore and

quencher in close proximity, sufficient for quenching. Tyagi et al. at col. 6, ll. 20-26, col. 9, ll. 59-65, and Figure 1. The interactive label moieties are separated when one or both of the target complementary regions (i.e., probes) is bound to the target nucleic acid.

According to Tyagi et al., the choice of whether to use a unimolecular or bimolecular probe depends upon the particular conditions under which the target nucleic acid is to be detected. Here, Tyagi et al. is very clear.

> [F]or assays that include a step or steps that may separate the affinity pair in a target-independent manner, only unimolecular probes are suitable.
>
> Tyagi et al. at col. 6, ll. 32-34 (emphasis added).

Tyagi et al. elaborates on the prohibition against the use of bimolecular probes:

> Bimolecular probes, as stated above, are not suitable for use in any reaction, e.g., PCR, in which the affinity pair would be separated in a target-independent manner.
>
> Tyagi et al. at col. 6, l. 67 through col. 5, l. 3 (emphasis added).

On the desirability of using unimolecular probes for amplification reactions including PCR, Tyagi et al. teach:

> Unimolecular probes with interactive labels according to the invention are particularly useful in assays for tracking polymerase chain reactions, since the probes according to this invention can open and close with a speed that is faster than the speed of thermal cycling.
>
> Tyagi et al. at col. 22, l. 66 through col. 23, l. 3 (emphasis added). See, also, Tyagi et al. at col. 20, ll. 47-49.

Tyagi et al. provide a sound scientific rationale supporting their admonition against the use of bimolecular probes in amplification reactions. Tyagi et al. teach:

> These strand displacement probe complexes have drawbacks. The mechanisms is two-step, in that the probe complex must first bind to the target and then strand-displacement, via branch migration, must occur before a target is recognized and a signal is generated. Bimolecular probe complexes are not reported to form with high efficiency, resulting in probe preparations wherein the majority of the target binding regions may not be annealed to a labeled strand. This may lead to competition between label-bearing and label-free target binding regions for the same target sequence… Moreover, the displaced labeled strand may need to be separated from the unhybridized probe complexes before a signal may be detected.
>
> Tyagi et al. at col. 3, ll. 11-24 (emphasis added).

Thus, the teachings of Tyagi et al. are clear: bimolecular probes should not be used in assays, such as PCR, in which the probes may be separated in the absence of target nucleic acid. This is a clear teaching away from Appellant's claimed method because the claimed method requires the use of bimolecular probes for monitoring an amplification reaction. The Examiner has not acknowledged or addressed this teaching away which Appellant submits vitiates the alleged motivation to combine Heid and Heller, and establishes the non-obviousness of the instant invention.

2.4. Di Cesare and/or Hiroaki fail to remedy the deficiencies of Heid et al. and Heller

Claims 11, 14-18, and 30-35 stand finally rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Heid in view of Heller and as evidenced by Mullis, as applied above, and in further in view of Di Cesare et al. (U.S. Patent No. 5,716,784; "Di Cesare") or Hiroaki et al. (EP 0461 863 A1; "Hiroaki").

The Examiner applies Heid and Heller as above but acknowledges that they fail to disclose a complementary probe pair having the specified difference in melting temperature—allegedly remedied by Di Cesare—and HCV-specific sequences for targets, primers, and

probes—allegedly remedied by Hiroaki. Neither Di Cesare nor Hiroaki disclose using a multi-probe detection system for real-time PCR, remedies the incompatibilities of Heid and Heller, or otherwise motivate the artisan to combine these prior art references, nor are they alleges for such purposes. Accordingly, Appellant submits that the rejection of claims 11, 14-18, and 30-35 is traversed and should be withdrawn.

CONCLUSION

For the reasons discussed above, Appellant respectfully submits that all rejections of claims 2-9, 11, and 14-38 are in condition for allowance, and respectfully request that the rejections be withdrawn or reversed, and that the claims be allowed to issue.

Respectfully submitted,

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APPENDIX A: CLAIMS APPENDIX

1. (Cancelled).

2. (Previously presented) The method of claim 20 wherein the amplification is carried out using a thermostable nucleic acid polymerase.

3. (Previously presented) The method of claim 20 wherein the fluorophore on the first probe and the quencher molecule on the second probe are on complementary base pairs.

4. (Previously presented) The method of claim 20 wherein the fluorophore and quencher molecules are within about 1 to 3 hybridized base pairs of each other.

5. (Previously presented) The method of claim 20 wherein the fluorophore and quencher molecules are within 3 or more hybridized base pairs of each other.

6. (Previously presented) The method of claim 20 wherein the fluorophore is on the 5' terminal nucleotide of the first probe and the quencher is on the 3' terminal nucleotide of the second probe.

7. (Previously presented) The method of claim 20 wherein the fluorophore is on the 3' terminal nucleotide of the first probe and the quencher is on the 5' terminal nucleotide of the second probe.

8. (Previously presented) The method of claim 20 wherein the second probe is shorter than the first probe.

9. (Previously presented) The method of claim 8 wherein the second probe is at least three nucleotides shorter than the first probe.

10. (Cancelled).

11. (Previously presented) The method of claim 20 wherein the first and second probes have a dissociation temperature difference of 2 degrees or more.

12-13. (Cancelled).

14. (Previously presented) The method of claim 20 wherein the first probe has the sequence of SEQ ID NO. 3.

15. (Previously presented) The method of claim 20 wherein the first probe has the sequence of SEQ ID NO. 4.

16. (Previously presented) The method of claim 20 wherein the amplification method is the polymerase chain reaction and wherein a primer for use in the polymerase chain reaction has the sequence of SEQ ID NO. 1.

17. (Previously presented) The method of claim 20 wherein the amplification method is the polymerase chain reaction and wherein a primer for use in the polymerase chain reaction has the sequence of SEQ ID NO. 2.

18. (Previously presented) The method of claim 20 wherein the target nucleic acid comprises the hepatitis C virus genome or segment thereof.

19. (Previously presented) The method of claim 20 wherein the method of amplification is selected from the group consisting of polymerase chain reaction, ligase chain reaction, gap ligase chain reaction, transcription mediated amplification, nucleic acid sequence based amplification and strand displacement amplification.

20. (Previously presented) A method for real-time monitoring of nucleic acid amplification comprising:

amplifying a target nucleic acid and monitoring said target nucleic acid during said amplification using a first oligonucleotide probe and a second oligonucleotide probe,

said first probe;

i) is capable of hybridizing to said target nucleic acid;

ii) comprises a fluorophore; and

iii) is not equal in length to said second probe;

said second probe;

i) is capable of hybridizing to said first probe; and

ii) has a quencher molecule which quenches said first probe fluorophore when said first and second probes are hybridized to each other; and

detecting fluorescence of said first probe fluorophore in real-time to monitor amplification, wherein an increase in fluorescence correlates with amplification.

21. (Previously presented) The method of claim 20 wherein the amplification method includes the use of a primer pair that flanks the first and second probe.

22. (Previously presented) The method of claim 20 wherein the longer probe binds preferentially to the target polynucleotide and when preferentially bound to the target polynucleotide the fluorescence intensity of the fluorophore is greater than the fluorescence intensity of the fluorophore when hybridized to the second probe.

23. (Previously presented) A method for monitoring nucleic acid amplification comprising:

(i) amplifying a target nucleic acid in a cycling amplification reaction in the presence of a first probe and a second probe;

said first probe;

i) is capable of hybridizing to said target nucleic acid;

ii) comprises a fluorophore; and

iii) is not equal in length to said second probe;

said second probe;

i) is capable of hybridizing to said first probe; and

ii) has a quencher molecule which quenches said first probe fluorophore when said first and second probes are hybridized to each other; and

(ii) assessing the amount of amplified target nucleic acid produced by said amplification reaction by detecting the amount of fluorescence of said first probe fluorophore during a plurality of cycles of said amplification reaction, wherein the amount of fluorescence correlates with the amount of amplified target nucleic acid.

24. (Previously presented) The method of claim 23 wherein the fluorophore on the first probe and the quencher molecule on the second probe are on complementary base pairs.

25. (Previously presented) The method of claim 23 wherein the fluorophore and quencher molecules are within about 1 to 3 hybridized base pairs of each other.

26. (Previously presented) The method of claim 23 wherein the fluorophore is on the 5' terminal nucleotide of the first probe and the quencher is on the 3' terminal nucleotide of the second probe.

27. (Previously presented) The method of claim 23 wherein the fluorophore is on the 3' terminal nucleotide of the first probe and the quencher is on the 5' terminal nucleotide of the second probe.

28. (Previously presented) The method of claim 23 wherein the second probe is shorter than the first probe.

29. (Previously presented) The method of claim 28 wherein the second probe is at least three nucleotides shorter than the first probe.

30. (Previously presented) The method of claim 23 wherein the first and second probes have a dissociation temperature difference of 2 degrees or more.

31. (Previously presented) The method of claim 23 wherein the first probe has the sequence of SEQ ID NO. 3.

32. (Previously presented) The method of claim 23 wherein the first probe has the sequence of SEQ ID NO. 4.

33. (Previously presented) The method of claim 23 wherein the amplification reaction is the polymerase chain reaction and wherein a primer for use in the polymerase chain reaction has the sequence of SEQ ID NO. 1.

34. (Previously presented) The method of claim 23 wherein the amplification reaction is the polymerase chain reaction and wherein a primer for use in the polymerase chain reaction has the sequence of SEQ ID NO. 2.

35. (Previously presented) The method of claim 23 wherein the target nucleic acid comprises at least a segment of the hepatitis C virus genome.

36. (Previously presented) The method of claim 23 wherein the method of amplification is selected from the group consisting of polymerase chain reaction, ligase chain reaction, gap ligase chain reaction, transcription mediated amplification, nucleic acid sequence based amplification and strand displacement amplification.

37. (Previously presented) The method of claim 23 wherein the amplification reaction includes the use of a primer pair that flanks the first and second probe.

38. (Previously presented) The method of claim 23, wherein the fluorescence of said first probe fluorophore is detected during every cycle of said amplification reaction.

APPENDIX B: EVIDENCE APPENDIX

1. Di Cesare et al., U.S. Patent 5,716,784

2. Heid et al. Genome Res. 6:986-994 (1996).

3. Heller, U.S. Patent 5,565,322

4. Hiroaki et al., European Patent Publication EP 0461863.

5. Mullis et al., U.S. Patent 4,965,188

6. Tyagi et al., U.S. Patent 6,103,476

APPENDIX C: RELATED PROCEEDINGS APPENDIX

None.